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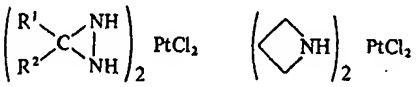
In the Search for New Anticancer Drugs. VII.*

Platinum Complexes of Diaziridines and Azetidine

G. Sosnovsky and J. Lukszo

Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, WI 53201, USA

Summary. Three new diaziridine platinum(II) complexes *(4a)–(4c)*, and a new azetidine platinum(II) complex *(3b)* were synthesized and tested against the lymphocytic leukemia P388 in mice.



(4a) $R^1=R^2=CH_3$
(4b) $R^1=R^2=C_2H_5$
(4c) $R^1R^2=-/CH_2/_5-$

(3b)

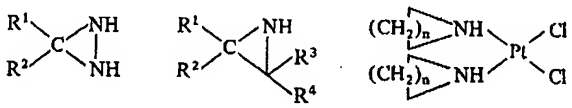
Moderate to good activity was found for all compounds as evidenced by a T/C value of 162 at a dose of 32 mg/kg for *(3b)*, 190 at a dose of 32 mg/kg for *(4a)*, 139 at a dose of 4 mg/kg for *(4b)*, and 142 at a dose of 20 mg/kg for *(4c)*.

Key words: Cisplatin – Diaziridine and azetidine platinum complexes – Leukemia P388 – Mice

Introduction

The discovery of the potent anticancer agent *cis*-dichlorodiammine platinum(II), or cisplatin (Rosenberg et al. 1969), has been followed by extensive clinical testing of this compound, and as a result, this drug is presently used in the treatment of various human cancers (Carter et al. 1981; Pouskoulelı and Kourounakis 1980). Unfortunately, cisplatin has been shown to have various dose-limiting side-effects (Carter et al. 1981), such as nephrotoxicity (Stark and Howell 1978). Consequently, many attempts have been made over the years to synthesize other platinum metal complexes in the hope of finding safer and more effective cisplatin analogs (Connors et al. 1972; Tobe and Khokhar 1977; Bradner et al. 1980; Prestayko et al. 1979).

In our search for new anticancer agents among the derivatives of small heterocyclic systems we recently reported (Sosnovsky and Lukszo 1983) the synthesis and in vivo evaluation in lymphocytic leukemia P388 of several phosphorylated diaziridine derivatives. Although these compounds were found to be inactive, a close structural resemblance between the diaziridines (1) and aziridine (2) ring systems, and the reported anticancer activity of compound *(3a)* (Connors et al. 1972; Tobe and Khokhar 1977) prompted us to synthesize and evaluate in vivo the platinum(II) complexes *(4a)–(4c)* which contain diaziridine moieties.



(1) *(2)*

(3a) n=0
(3b) n=1
(3c) n=2
(3d) n=3
(3e) n=4

In addition, we have also prepared and tested in vivo the azetidine platinum(II) complex *(3b)* which has not been reported to date. Compound *(3b)* represents the missing link in the series of platinum(II) complexes *(3a)–(3e)* derived from secondary cyclic amines reported to posses anticancer activity against the lymphoid leukemia L1210 and PC6 mouse plasma cell tumor (Connors et al. 1972; Tobe and Khokhar 1977).

Materials and Methods

Mice

Male mice CD_2F_1 (for testing; aver age weight 18–21 g) and DBA/2 (for tumor propagation; Geran et al. 1972) 6–7 weeks old were sup-

Offprint request to: Prof. George Sosnovsky (address see above)

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plied by Hawley Sprague Dawley (Indianapolis, Ind). Mice were fed rodent laboratory chow 5001 (Ralston Purina Co.) and water ad libitum.

Drugs

Compounds were administered in the form of a water suspension, with Tween 80 (Sigma Co.) used as a dispersing agent

Antitumor Testing

Antileukemic experiments were initiated on day 0 by IP implantation into the male CD_2F_1 mice of 10^6 P388 acites cells according to the NCI protocol (Geran et al. 1972). Drug treatment was commenced on day 1 and consisted of nine daily IP injections (days 1–9). Experiments were terminated when no mice remained alive. A single-dose injection of 5-fluorouracil, 200 mg/kg, was included as a positive control, resulting in a T/C value exceeding 135, as specified by the NCI protocol. All-treated groups consisted of six mice and the leukemia group consisted of eight mice. The mice were observed daily, and the antileukemic activity of each compound was compared on the basis of the T/C criterion, where T represents the mean survival time of the treated group and C the mean survival time of the tumor-bearing control group. A value of $T/C \geqq 125$ is usually considered to be the minimum requirement for a compound to be considered as active (Geran et al. 1972). The percent increase in life-span (ILS%) was calculated from the formula $[(T-C)/C] \times 100$. Clearly, the larger the value of the ILS, the more promising is the compound as an anticancer drug.

Analytical Procedures

All melting points (dec.) were obtained with a Thomas Hoover melting point apparatus, model 6406-K using a calibrated thermometer. The IR spectra were recorded on a Perkin-Elmer spectrophotometer, model 735 B. Mass spectra were recorded on a Hewlett-Packard mass spectrometer, model 5985 GS, using a direct insertion probe, a source pressure of 2×10^{-7} torr, and methane as the reactant gas. Therefore, M^+1 values are reported. Microanalyses were performed either on an F&M Scientific Corporation Carbon, Hydrogen, Nitrogen Analyzer, model 185, or by the Atlantic Microlab, Inc. Atlanta, Georgia. ^{13}C NMR spectra were recorded on a Bruker WM-250 NMR Spectrometer. All diaziridines and azetidine used in this work were prepared by methods known from the literature; *(1a)*, *(1c)* by that of Schmitz and Ohme (1961), *(1b)* by that of Paulsen and Huck (1961), and *(6)* by that of Wadsworth (1973).

Synthesis

Preparation of *cis*-Dichlorobis(azetidine) Platinum(II) *(3b)*.

A solution of potassium tetrachloroplatinate(II) (622.4 mg 1.50 mmol) in water (4.0 ml) was combined with a solution of azetidine *(6)* (180.0 mg, 3.15 mmol) in water (1.5 ml), and the resultant solution stirred for 24 h at 20–25 °C. During this period a pale yellow precipitate was found. The precipitate was collected by filtration and washed successively with water (2×0.5 ml), 6 *N* hydrochloric acid (1.5 ml), methanol (2×1.0 ml), and ethyl ether (2×2 ml). Drying of the solid at 20 °C/1 torr gave 395.0 mg (69%) of *(3b)* which gradually turns dark above 145 °C, and melts with decomposition at 161–164 °C.

MS m/e: 378(63), 379(69), 380(100), 381(55), 382(64), 383(17), 384(21).
IR (KBr): $\nu = 910$, 1380, 1440, 2950, 3130 cm^{-1}.
$C_6H_{14}Cl_2N_2Pt$: calculated, C 18.95% H 3.71% N 7.37% (380.32) found C 18.76% H 3.68% N 7.28%.

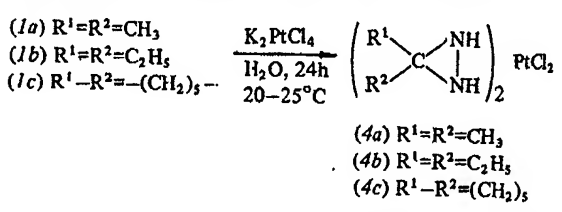
Preparation of *cis*-Dichlorobis-(3,3-dimethyldiaziridine) Platinum(II) *(4a)*,
cis-Dichlorobis-(3,3-diethyldiaziridine) Platinum(II) *(4b)*, and *cis*-Dichlorobis-[3,3-(1,5-pentanediyl)diaziridine] Platinum(II) *(4c)*

General procedure. A solution of potassium tetrachloroplatinate(II) (415.0 mg, 1.0 mmol) in water (2 ml) was combined with a solution of either 3,3-dimethyldiaziridine *(1a)* (151.0 mg, 2.1 mmol), 3,3-diethyldiaziridine *(1b)* (210.0 mg, 2.1 mmol) or 3,3-(1,5-pentanediyl)-diaziridine *(1c)* (235.0 mg, 2.1 mmol) in water (1 ml), and the resultant solution stirred for 24 h at 20–25 °C. During this period a pale yellow precipitate was formed in each case. The precipitate was collected by filtration and washed with cold water (0 °C, 2×0.5 ml) and ethyl ether (2×0.5 ml). Drying of the solids at 20 °C/1 Torr gave the yellow, crystalline products *(4a)–(4c)*. *(4a)* 210.0 mg (51%), m.p. 151–154 °C (dec.).

MS m/e: 409(9), 410(10), 411(11), 412(9), 413(9), 415(9), 416(9), 417(9).
IR (KBr): $\nu = 810$, 1110, 1300, 1380, 3130 cm^{-1}.
^{13}C NMR (DMSO–d_6): 60.40 and 59.58 ppm.
$C_6H_{16}Cl_2N_4Pt$: calculated, C 17.56% H 3.93% N 13.65% (410.36) found C 16.28% H 3.60% N 11.76%.
(4b) 402.0 mg (86%), m.p. 152–157 °C (dec.).
IR (KBr): $\nu = 860$, 1380, 1450, 2950, 3120 cm^{-1}.
^{13}C NMR (DMSO–d_6): 66.01, 66.06 and 66.18 ppm.
$C_{10}H_{24}Cl_2N_4Pt$: calculated, C 25.75% H 5.19% N 12.01% (466.46) found C 24.81% H 4.89% N 11.49%.
(4c) 441.0 mg (90%), m.p. 153–5 °C (dec.).
IR (KBr): $\nu = 890$, 1310, 1380, 1440, 2900, 3110 cm^{-1}.
^{13}C NMR (DMSO–d_6): 64.20, 63.77, 63.67, and 56.94 ppm.
$C_{12}H_{24}Cl_2N_4Pt$: calculated, C 29.39% H 4.93% N 11.42% (490.48) found C 29.42% H 4.97% N 11.37%.

Results and Discussion

As starting materials three readily available diaziridines, *(1a)–(1c)* (Schmitz and Ohme 1961; Paulsen and Huck 1961) bearing no substituents at the nitrogen atoms were chosen. These diaziridines were reacted in an aqueous solution with potassium tetrachloroplatinate(II) at 20–25 °C in accordance with the general literature procedure (Connors et al. 1972). After 24 h of stirring of the reaction mixtures the yellow precipitates were collected by filtration, washed with water and ethyl ether and dried in vaccum.

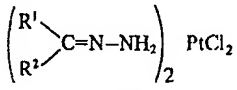


(*4a*) $R^1=R^2=CH_3$
(*4b*) $R^1=R^2=C_2H_5$
(*4c*) $R^1-R^2=(CH_2)_5$

In contrast to the literature procedure (Connors et al. 1972), compounds *(4)* were not washed with hydrochloric acid because of their solubility in hydrochloric acid. This solubility seems to suggest that one of the two nitrogen atoms is bound to platinum while the other is involved in the protonation process.

The lower solubility of compounds *(4a)–(4c)* in water corresponds to the increase in molecular weight of the diaziridine moiety *1*. Compounds *(4a)* *(4c)* were prepared in a 51%, 86%, and 90% yield, respectively. The complexes *(4a)–(4c)* had very similar decomposition points above 150 °C, undergoing darkening over a range of 10–15 deg. C prior to decomposition.

To determine whether the diaziridine rings were still intact, complexes *(4a)–(4c) were analyzed by* ^{13}*C-NMR spectroscopy in dimethylsulfoxide-*d_6. *All of the compounds 4a–c* exhibited the most downfield set of peaks in the region of 57·66 ppm attributed to the diaziridine ring carbon atom. These values closely match the values of 56–69 ppm which were found for several diaziridine derivatives (Sosnovsky and Lukszo 1983). The reasons for the observation of sets of peaks instead of single peaks are (a) the presence of several platinum isotopes; and (b) various interactions of dimethylsulfoxide with the platinum complexes (Kerrison and Sadler 1977). Nevertheless, a lack of peaks at a lower field than 56·69 ppm seems to be adequate evidence for the intact diaziridine ring in *(4)*, since an open-chain product such as *(5)* would have a peak corresponding to the sp^2 carbonyl carbon at a considerably lower field than 56–69 ppm (Sosnovsky and Lukszo 1983).

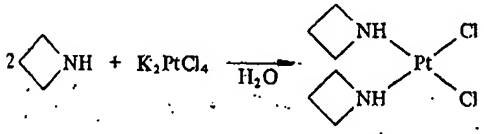


(5)

The microanalysis matched well the calculated values for *(4c)*, whereas in the case of *(4b)* and *(4c)* lower values than expected were repeatedly obtained.

Mass spectrometry of compound *(4a)* resulted in a set of lines of parent peaks corresponding to combinations of four major platinum and two chlorine isotopes. In the case of *(4b)* only fragments devoid of chlorine atoms and of 15 mass units, such as, CH_3 or NH, were observed. Because of low volatility of compound *(4c)* no mass spectrometric analysis could be achieved.

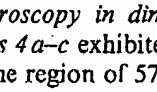
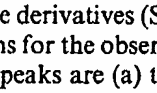
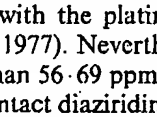
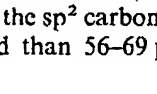
Cis-Dichlorobis(azetidine) platinum(II) *(3b)* was synthesized similarly to the preparation of compounds *(4a)–(4c)* in accordance with the general procedure (Connors et al. 1972).



(6) (3b) n=1

In contrast to the diaziridine complexes *(4a)–(4c)*, the azetidine complex *(3b)* was not solubilized by addition of hydrochloric acid to the aqueous suspension of *(3b)*. The compound was readily characterized by microanalysis, infrared spectroscopy, and mass spectrometry (see *Synthesis)*.

Table 1. In vivo evaluation of azetidine *(3b)* and diaziridine platinum (II) complexes *(4a–c)* for antitumor activity

Compound	Daily dose (mg/kg)	T/C (%)	ILS (%)
 *(3b)*	4	127	27
	8	149	49
	16	149	49
	32	162	62
 *(4a)*	8	155	55
	16	186	86
	32	190	90
 *(4b)*	2	115	15
	4	139	39
	8	131	31
	16	137	37
 *(4c)*	5	120	20
	10	113	13
	20	142	42
	40	82	−18

The new azetidine derivative *(3b)* possesses a moderate antitumour activity, as evidenced by the T/C value of 162 at 32 mg/kg. Unfortunately, this result cannot be directly compared with the results reported for other compounds of the same series *(3)* (Connors et al. 1972; Tobe and Khokhar 1977), because of the use of different tumors, L1210 and PC6, and different protocols. Among the three diaziridine Platinum(II) complexes, compound *4a* possesses good anticancer activity, as evidenced by the T/C value of 190 at 32 mg/kg, whereas compounds *(4b)* and *(4c)* have only modest acitivity, with T/C = 139 at 4 mg/kg and T/C = 142 at 40 mg/kg.

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